DEDICATION

To my mother and to the soul of my father

To my dearest wife,my beloved, kid Hamed,

To My sisters and brothers

"To all of them I dedicate this work"

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I would like hereby to thank all my colleagues, without their sincere efforts, this research would have never come to accomplished.

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ABSTRACT

Background: *Tuberculosis* remains a major global health problem. Lymph nodes represent the most common site for extra pulmonary *tuberculosis*. Several techniques have been employed for the diagnosis of extrapulmonary*Tuberculosis* (EPTB) including; conventional Hematoxylin and Eosin stain, the Ziehl-Neelsen(ZN) stain, Immunohistochemistry(IHC) and Polymerase chain reaction (PCR). Each of these techniques has advantages and limitations.

Aim: To evaluate the diagnostic utility of PCR, IHC and ZN stain in detection of *Mycobacterium tuberculosis* in histological sections.

Materials and methods: This is descriptive analytical cross sectional study conducted in Khartoum State during the period from July 2012 to July 2015. In this study 161lymph nodes tissue biopsies were used in this study. These 161 samples were reinvestigated by H and E. The specific monoclonal anti 38KD was used to detect *mycobacterium tuberculosis* (MTB) in histological section by IHC, and IS6110 sequence was used to detect MTB by PCR. IS6110 PCR assay was performed in comparison with the H&E, ZN stain and IHC.

Results: In this study, 161 enlarged lymph nodes were diagnosed as having lymph node*tuberculosis* by histopathology. The minimum age of study population was 4 years and the maximum was 85 years with a mean age of 51 years. The study population was divided in to two group pediatric 42 (26%) and adult 119 (74%). The male female ratio was 0.89:1.11.The study population subsequently subdivided in to other groups started from >8 years up to 51+. The great majority of the specimens were obtained from cervical lymph node which representing 100 (62%), followed by axillary lymph node representing 17 (11%).The other sites include mediastinal,

mesenteric, inguinal, and submandibular, constituting 10 (6%), 7 (4%), 7 (4%), and 4 (3%) respectively. The study populations were further divided into two groups according to the presences of strong and weak *tuberculosis*histopathological evidences. Accordingly, of the 161 cases, 118 (73.3%) were categorized as having strong evidences and the remaining 43(26.7) were detected with weaker evidences, cases.

In this study, of 161 studied lymph nodes, only 4 (2.5%) were positive with ZN and 157(97.5%) were negative. These 4(2.5%) ZN positive cases were previously found as strong evidence, while the remaining 114 strong evidences cases were negative with ZN stain. Statistically, no significant association between TB histopathologicalevidences and ZN stain, *P-value=0.221*.

IHC monoclonal anti 38-KD was positive in 129 (80%) of cases, the remaining 32 (20%) were negative with IHC. Of the entire 129 IHC positive cases, 100 (62%) cases were identified as strong evidences cases and the remaining 29 (18%) were at weak level. Statistically, IHC stain is significantly associated with TB histopathologicalevidences *P-value = 0.015.*

In this study, of 161 studied lymph nodes, 135 (84%) were positive for IS1160 PCR and the remaining were negative, from the entire 135 (84%) PCR positive cases, 106 cases were previously found as strong evidence and 29 was found weak evidence. Of remaining 26 (16%) PCR negative cases, only 12 specimens were showed strong evidence for TB, and 14 were weak evidence, statistically, PCR is significantly associated with TB histopathologicalevidences, *P-value*=0.001. On the other hand PCR was used as a gold standard for comparing the other variables, accordingly the sensitivity and specificity of histopathology diagnosis & ZN stain were

78.5%, 46.1% and 3.0%, 100% respectively. In contrast the sensitivity and specificity of anti 38KD IHC was 95.5%, 100% respectively.

Conclusion: IHC with monoclonal anti 38KD and PCR with IS6110 oligonucleotides are rapid, sensitive, and specific methods for establishing the diagnosis of *tuberculosis* in histologic specimens. Immunohistochemistry has the advantages over PCR of being robust, quicker, and cheaper, and it can be used in high-endemic countries.

ملخص الدراسة

خلفية:يعتبرمرض الدرن مشكلة صحية عالمية رئيسية. تمثل العقداليمفاويةالموقع الأكثرشيوعاًلمرض الدرن خارج الرئة،وقداستخدمت العديدمن التقنيات لتشخيص مرض الدرن خارج الرئة،بمافي ذلك،صبغةالهيماتوكسلينوالايوسن(H and E) التقليدية،صبغة زيل- نيلسن (ZN)،كيمياءالأنسجةالمناعية (IHC) وتفاعل البوليميريزالمتسلسل(PCR) للتحقق من وجودمرض الدرن.

الهدف: كان الهدف من هذه الدراسةهوتقييم الفائده التشخيصية لتفاعل البوليميريزالمتسلسل،كيمياءالأنسجةالمناعية وصبغةزيلنيلسن في الكشف عن المتفطرة الدرنية فى القطاعات النسيجية.

الموادوالطرق:هذه دراسةارتجاعية تحليلية أجريت في ولاية الخرطوم خلال الفترة من يولو عام 2012 إلى يوليو 2015. فى هذه الدراسة 161عقده ليمفاوية تم استخدامها و أعيد تسخيصيها بصبغة الهيماتوكسلينوالايوسن.

في هذه الدراسة،استخدم الجسم المضادوحيدالنسل (38-KD) عن طريق تقنية كيمياءالأنسجةالمناعية فى الكشف عن المتفطره الدرنية فى المقاطعالنسيجية،وايضاً استخدم التسلسل (IS6110) عن طريق تقنية البوليميريزالمتسلسل في الكشف عن المتفطرة الدرني فى تلك الانسجة. وتمت مقارنة البوليميريزالمتستلسل بالتشخيص النسيجى،صبغةزيل-نيلسن و تقنيةكيمياءالانسجةالمناعية.

النتائج: في هذه الدراسة الوصفيه الارتجاعية كان هنالك عدد 161 مريض مصاب بتضخم العقد الليمفاوية. وتم تشخيصهم عن طريق التشخيص النسيجي على ان لديهم مرض الدرن فى العقد الليمفاوية.اظهرت التحاليل الاحصائية أن أقل أعمارالمرضي الذين شملتهم الدراسةكان 4 سنوات واعلاها 80 سنة بمتوسط عمر 51 سنة. تم تقسيم المرضي الى مجموعتين،أطفال وكان عددهم 42(26%) وكبارو كان عددهم 119(74%)،وكانت نسبةالذكور للانإناث 0.89 : 1.11. لاحقاً تم تقسيم المرضي الى مجموعات اخري تبداء بأقل من عمر 8

سنوات وتنتهي بأكثر من 51 سنة.فى هذه الدراسةمعظم العينات تم الحصول عليها من العقد الليمفاوية السطحية ومثلت 100(42%)عقدة ليمفاوية،تبعتهاالعقداللليمفاويةالأبطية ومثلت 17(11%)،العقداللليمفاويةالأخري مثل العقدة الليمفاوية المنصفية،العقدةالليمفاوية المساريقية،العقدةالليمفاويةالأربية والعقدةالليمفاويةتحت الفك السفلي مثلت 10(6%)، 7(4%)، 4(3%) على التوالي.أيضاًتم تقسيم المرضي لاحقاً الى مجموعتين أعتماداً على وجود الدلائل القوية والضعيفة لمرض الدرن،وأعتماداًعلي ذلك،من 161 حالةكان هنالك 118(73.3%) حالةأعطت دلائل قوية لوجودمرض الدرن،والمتبقية 43(26.7%) ذات دلائل ضعيفة لمرض الدرن.

فى هذه الدراسة،من عدد 161 عقدة لميفاوية تمت دراستها بواسطة صبغة زيل-نيلسن،وكان هنالك 4(2.5) عقدة ليمفاوية أعطت نتائج موجبة لمرض الدرن، والمتبقي 157(97.5%) اعطت نتائج سالبة. هذه الأربعة(2.5%) حالات الموجبة لصبغة زيل-نيلسن كانت فى السابق ذات دلائل قوية لوجود مرض الدرن،والمتبقى 114 من الحالات القوية الدلائل لمرض الدرن أعطت نتائج سالبة لصبغة زيل-نيلسن.

كانت كيمياء الأنسجة المناعية بأستخدام الجسم المضاد وحيد النسل (KD-38) موجبةفى 129(80%) حالة،والمتبقية 32(20%) حالةكانت سالبة. ومن 129 حالة موجبة،كانت هنالك 100(62%) حالة ذات ادلة قوية لوجود المرض و 29(18%) ذات أدلة ضعيفة لوجود المرض. إحصائياًتقنية كيمياءالأنسجة المناعية لها ارتباط كبيرمع دلائل مرض الدرن،حيث كانت القيمه الاحتماليه = *0.015*.

فى هذه الدراسةأيضاً ،من 161 عقدة لمفاوية تمت دراستها،كانت هنالك 135(84%) حاله موجبة لتقنيةالمبوليميريزالمتسلسل والمتبقية كانت سالبة. ومن 135 حالةموجبة،كانت هنالك 106 حالة وجدت سابقاً ذات أدله قوية لوجود مرض الدرن و29 حاله ذات أدله ضعيفة. إحصائياً تقنيةالبوليميريزالمتسلسل لديهاارتباط كبيرمع دلائل مرض الدرن،حيث كانت القيمةالإحتمالية = *0.001*.

من ناحية اخري تم استخدام البوليمريزالمتسلسل كمعيار ذهبي لقياس المتغيرات الأخري. وعليه كانت حساسية وخصوصية صبغة زيل-نيلسن وصبغة الهيماتوكسلينوالايوسن 3.0%،100%و 78.5%، 46.1% على التوالي،وفي المقابل حساسية وخصوصية كيمياء الانسجة المناعية 95.5%، 100%.

الخاتمه: تقنيةكيمياءالانسجةالمناعيةبأستخدام الجسم المضاد وحيدالنسل(KD-38) وتقنيةالبوليميريزالمتسلسل باستخدام التسلسل (IS6110) هي طرق سريعة،حساسة،وذات خصوصية في تشخيص المتفطره السلية فى العينات النسيجية. كيمياءالانسجة المناعية لديها مزايا أكثرمن البوليمريزالمتسلسل،كونهاقوية،أسرع،وأرخص، ويمكن استخدامها في البلدان المستوطنة.

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3- IhabHamedNourein, HussainGadelkarim Ahmed, Hassan Elsiddig Hassan, Mohammed AyedHuneif, Abdelrahman Mohamed Abdelrahman, AbuobiedaBallaAbusharib.***"Monoclonal Anti 38-KD Immunohistochemistry: A Novel Method for Improving the Diagnosis of Pediatric Tuberculous Lymphadenitis."*** *International Journal of Science and Research (IJSR)* 3, no. 10. (2014): 88-91. www.ijsr.net/v3i10.php.

List of abbreviations

Abbreviations	Title
AC	Adsorption column
ADIS	Acquired Immunodeficiency Syndrome
AFB	Acid fast bacilli
BAL	Bronchial Aspiration Lavage
BB	Bronchial Brushings
BCG	Bacilli Calmette–Guérin
BW	Bronchial Washings
CD	Cell Diffrentiation
CT	Computerized tomography
DAB	Diaminobenzidine
DNA	Deoxyribo nucleic acid
DPX	Distrene, Plasticiser, Xylene
EB	Elution buffer
EPTB	Extrapulmonary*tuberculosis*
ESAT-6	Early-secreted antigenic target-6
FFPE	Formalin- fixed, paraffin- embedded
FISH	Fluorescence in situ hybridization
FNAC	Fine needle aspiration cytology
H and E	Hematoxylin and Eosin
HIV	Human Immunodeficiency Virus

HRP	Horse reddish peroxidase
IGRAs	Interferon-gamma release assays
IHC	Immunohistochemistry
IL	Interlukin
ISH	In situ hybridization
KD	Kilo Dalton
LJ	Lowenstein–Jensen
LN	Lymph node
LNTB	Lymph node *tuberculosis*
MRI	Magnetic resonance imaging
MTB	Mycobacterium *tuberculosis*
MTC	Mycobacterium *tuberculosis* complex
NAA	Nucleic acid amplification
NK	Natural Killer
NTM	Non-tuberculous mycobacteria
PBS	Phosphate buffer Saline
PBS	Post bronchoscopy sputum collection
PCR	Polymerase chain reaction
PPD	Purified Protein Derivative
PTB	Pulmonary *tuberculosis*
RNA	Ribo nucleic acid
RPM	Round per minute
SI	Sputum Induction
SPSS	Statistical Package for Social Sciences
TB	*Tuberculosis*
TBB	Transbronchial biopsy
TBE	Tris/Borate/EDTA

TBL	Tuberculous lymphadenitis
TNFα	Tumor Necrosis Factor α
TST	Tuberculin skin test
UV	Ultraviolet
WHO	World Health Organization
ZN	Ziehl-Neelsen